

## **AMENDMENTS TO THE CLAIMS:**

This listing of claims will replace all prior versions, and listings, of claims in the application.

### **LISTING OF CLAIMS:**

Claims 1 to 12 (Canceled)

13. (new) A method for purifying a polypeptide of interest, or a biomolecule complex comprising the polypeptide of interest, said method comprising:

- (a) providing a eukaryotic expression environment such that a fusion protein is expressed under conditions that allow formation of a complex between the fusion protein and one or more other biomolecules; said fusion protein comprising said polypeptide and at least two different affinity tags; and
- (b) purifying said polypeptide, or any said complex that forms, by performing a combination of at least two different affinity purification steps, each comprising binding the fusion protein, or a truncated fusion protein wherein one of the affinity tags is cleaved off, via one affinity tag to a support material capable of selectively binding one of the affinity tags, and separating the fusion protein or the truncated fusion protein or the polypeptide from the support material after substances not bound to the support material have been removed,

wherein any of said one or more other biomolecules which are bound to said polypeptide in any said complex remain associated with said polypeptide during said step (b), thereby purifying said polypeptide of interest or biomolecule complex comprising said polypeptide of interest.

14. (new) The method of claim 13 wherein at least one of said at least two different affinity purification steps comprises recovering the fusion protein or truncated fusion protein from said support material by elution.

15. (new) The method of claim 13 or 14 wherein at least one of said at least two different affinity purification steps comprises recovering the truncated fusion protein or

polypeptide by a method comprising cleaving off one or more of said affinity tags.

16. (new) The method of claim 15, wherein the Tobacco Etch Virus protease NIA is used to cleave off said one or more affinity tags.

17. (new) The method of claim 13 or 14 wherein said fusion protein is expressed intracellularly.

18. (new) The method of claim 13 or 14 wherein said at least two different affinity tags are all placed N-terminal, or are all placed C-terminal, to the polypeptide.

19. (new) The method of claim 13 wherein one of the at least two affinity tags consists of one or more IgG binding domains of protein A of *Staphylococcus aureus*.

20. (new) The method of claim 13 or 19 wherein one of the at least two affinity tags consists of one or more calmodulin binding peptides.

21. (new) The method of claim 13 wherein the fusion protein comprises a proteolytic cleavage site between two different affinity tags.

22. (new) The method of claim 13 wherein the fusion protein comprises the following components in the order stated, starting from the N- or the C-terminus,

- (a) one or more IgG binding domains of protein A of *Staphylococcus aureus*;
- (b) Tobacco Etch Virus protease NIA cleavage site;
- (c) one or more calmodulin binding peptides; and
- (d) said polypeptide of interest.

23. (new) The method of claim 22 wherein said performing comprises the following steps in the order stated:

- (i) binding of the fusion protein to IgG on a first support material;
- (ii) removing unbound substances;
- (iii) cleaving of the fusion protein with the Tobacco Etch Virus protease NIA to produce a truncated fusion protein;

- (iv) binding of the truncated fusion protein to calmodulin on a second support material;
- (v) removing unbound substances; and
- (vi) eluting the truncated fusion protein with a chelating agent.

24. (new) The method of claim 13 or 14 wherein the support material is a resin matrix packed in an affinity column.

25. (new) The method of claim 13 wherein the support material is a matrix formed by SEPHAROSE beads.

26. (new) The method of claim 13 or 14 wherein the fusion protein is expressed in a native form and is not overexpressed.

27. (new) The method of claim 13, 14, 22, or 23, which further comprises after step (b) a step (c) of detecting and/or purifying said one or more other biomolecules.

28. (new) The method of claim 27, wherein said step (c) comprises detecting and identifying said one or more other biomolecules using mass spectrometry.

29. (new) The method of claim 13, wherein said fusion protein comprises not more than two different affinity tags.

30. (new) The method of claim 13, wherein said purifying step comprises two different affinity purification steps.

31. (new) The method of claim 30, wherein said affinity purification steps comprise the following steps in the order stated:

- (i) binding of the fusion protein to a first support material;
- (ii) removing unbound substances;
- (iii) cleaving of the fusion protein to produce a truncated fusion protein;
- (iv) binding of the truncated fusion protein to a second support material;
- (v) removing unbound substances; and
- (vi) eluting the truncated fusion protein.

32. (new) The method of claim 13, 14, or 22, wherein said eukaryotic expression environment is a yeast cell.

33. (new) The method of claim 13, 14, or 22, wherein said eukaryotic expression environment is a mammalian cell.

34. (new) The method of claim 13, wherein said eukaryotic expression environment is a cell-free system.

35. (new) The method of claim 13, wherein said polypeptide is expressed in its natural host.

36. (new) The method of claim 13, wherein said one or more other biomolecules are proteins.

37. (new) The method of claim 36, wherein at least one of said one or more other biomolecules is a protein.

38. (new) The method of claim 13, wherein said step (b) comprises purifying said complex.